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The Paleoepidemiology of *Enterobius vermicularis* (Nemata: Oxyuridae) Among the Loma San Gabriel at La Cueva de los Muertos Chiquitos (600–800 CE), Rio Zape Valley, Durango, Mexico

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ABSTRACT: One hundred coprolites excavated from La Cueva de los Muertos Chiquitos (600–800 CE) in the Rio Zape Valley of present-day Durango, Mexico, were examined for the presence of helminth eggs utilizing standard archaeoparasitological techniques. Eggs of the human pinworm (*Enterobius vermicularis*) were recovered from 34 of the 100 coprolites examined. Eggs of parasites were photographed and measured before egg concentration values were calculated for each positive sample. Egg concentration values demonstrated an overdispersed pattern of distribution among the samples (66% uninfected, 25% less than 100 eggs/g, 8% between 100 and 500 eggs/g, and 1% more than 500 eggs/g). Given that only 5% of infected hosts in modern cases of human enterobiasis pass the eggs of parasites in their stools, the recovery of *E. vermicularis* eggs in 34% of the coprolites supports the conclusion that virtually all of the individuals utilizing the site during the coprolite depositional time frame likely were infected with this parasite. These data are discussed in light of other studies of prehistoric human enterobiasis.

KEY WORDS: archaeoparasitology, coprolite, Durango, Mexico, *Enterobius vermicularis*, La Cueva de los Muertos Chiquitos, paleoepidemiology, pathoecology, pinworm.

The pinworm, *Enterobius vermicularis*, is a common human parasite with evidence showing a host-parasite association going back as far as 8,000 years (Hugot et al., 1999). In a modern context, infection with this parasite occurs commonly among humans with prevalences among children, childcare workers, and institutionalized persons being reported as high as 50% (Burkhart and Burkhart, 2005). The oldest human pinworm eggs recovered from human remains in North America were reported from Danger Cave, an archaeological site in Utah that dates to about 10,200 calendar years ago (Rhode and Madsen, 1998; Goebel et al., 2007). Eggs identified as *E. vermicularis* have been recovered from many other North American sites that predate European contact of the thirteenth century AD, which suggests that this parasite was established in the Americas alongside their human hosts following migrations into the continent (Reinhard et al., 2016).

Evidence indicates that in parts of the prehistoric Americas, *E. vermicularis* was seemingly epidemic. Bearing in mind that only 5% of infected individuals release eggs in their stools, it is remarkable that pinworm eggs are often recovered from coprolites excavated from sites in the Greater Southwest in numbers exceeding 10% (Fry, 1974; Faulkner et al., 1989; Reinhard, 1992; Fugassa et al., 2011; Jiménez et al., 2012; Reinhard et al., 2016). Subsistence strategies and dwelling types have quantifiable effects on pinworm prevalences among ancient peoples (Hugot et al., 1999), and agricultural populations were more frequently found to have pinworms than hunter-gatherers, largely due to the impacts of sedentism (Reinhard, 1988). Ancient dwellings, many of which were built within caves, served as nidi for these parasites (Reinhard and Bryant, 2008), while the living habits of agriculturalists' were conducive to shifts in human parasitism in terms of both diversity and intensity of infection, which is reflected in archaeoparasitological analyses of coprolites (Reinhard, 1988).

Archaeoparasitological data can be utilized to examine the paleoepidemiology of ancient parasitism in ways similar to modern epidemiological studies (Han et al., 2003; Seo et al., 2008, 2014). Early paleoepidemiological studies of parasites began with Fry's examination of parasite prevalence in the Great Basin spanning nearly 10,000 years (Fry, 1977). Further examinations of the changes in parasitism related to Colorado Plateau hunter-gatherer/agriculturalist transitions were studied by Reinhard beginning a decade

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later (Reinhard, 1988, 1992), and paleoepidemiological studies in North American resumed only recently (Fugassa et al., 2011; Jiménez et al., 2012; Morrow and Reinhard, 2016).

In the present study, 100 coprolites excavated from La Cueva de los Muertos Chiquitos (CMC) were examined for the presence of *E. vermicularis* eggs. CMC is a cave located within the Rio Zape Valley, a region approximately 18 kilometers southeast of Guanaceví in Durango, Mexico (Jiménez et al., 2012; Morrow and Reinhard, 2016). This valley houses a series of caves once utilized by people of the Loma San Gabriel culture between 1,200 and 1,400 years ago (Brooks et al., 1962; Foster, 1986). This area represents an archaeological transition zone between the northernmost edge of Mesoamerica and the greater American Southwest (Kelley, 1956, 1971; Brooks and Brooks, 1980). Excavations of CMC recovered 21 skeletons of children, ranging between several months and three years of age, and more than 500 well-preserved coprolites (Brooks et al., 1962; Brooks and Brooks, 1978, 1980). These coprolites were sealed beneath adobe floors, which inhibited the influence of certain taphonomic agents, such as water, wind, flies, and scavengers (Morrow and Reinhard, 2016; Morrow et al., 2016).

Parasitism among the Loma San Gabriel has been predominately studied through the analysis of CMC coprolites (Jiménez et al., 2012; Cleeland et al., 2013; Morrow and Reinhard, 2016), and previous study of 36 CMC coprolites reported a 44% prevalence of *E. vermicularis* eggs (Jiménez et al., 2012). Herein, the recovery of *E. vermicularis* eggs from examinations of an additional 100 CMC coprolites are reported and the paleoepidemiology of this parasite at CMC is discussed.

MATERIALS AND METHODS

Coprolites from La Cueva de los Muertos Chiquitos (CMC) archived within the Pathoecology Laboratory in the School of Natural Resources at the University of Nebraska were used for the present study. This is a filtered-air, positive-pressure facility that is free of contaminants smaller than a micrometer. A total of 100 coprolites from this collection were given an individual analysis identification number, weighed, photographed, and placed into individually labeled plastic zippered bags. Many of these coprolites had been stored in the same bags as other coprolites, which gave these artifacts the potential to contaminate one another with the mechanical transfer of eggs, especially because these materials have been transported repeatedly since their excavation in the early 1960s. To reduce the retention of potentially contaminate eggs, the surfaces of each of the coprolites were abraded using cleaned, stiff brushes. To prevent modern contamination of material, nitrile gloves were worn during cleaning. Dusts from the abraded surfaces of these coprolites were retained in their primary analysis bags, and each coprolite was placed into a clean secondary analysis bag.

A subsample was extracted from each of the coprolites and weighed for standard analysis of parasites. These subsamples were rehydrated with 0.5% trisodium phosphate for 24 hr prior to disaggregation utilizing a magnetic stirring apparatus. The color of the rehydrated material was recorded. Samples were subsequently treated with 1 tablet/g of sample containing the spores of *Lycopodium* (Batch no. 124961, Department of Quaternary Geology, University of Lund, Lund, Sweden; containing approximately 12,500 spores/tablet). These tablets were dissolved using hydrochloric acid (HCl) in sterilized 50 ml plastic beakers and rinsed into each beaker of disaggregated material (Stockmarr, 1971).

The method for using *Lycopodium* to quantify the eggs of parasites from archaeological materials was first developed by Warnock and Reinhard (1992) and has become a standard quantification technique among archaeo- and paleoparasitologists (Martinson et al., 2003; Reinhard et al., 2008, 2012; Reinhard and Urban, 2003; Santoro et al., 2003; Sianto et al., 2005; Kumm et al., 2010; Fugassa et al., 2011; Jiménez et al., 2012; Searcey et al., 2013). By spiking samples with a known amount of *Lycopodium* spores, subsequent microfossil (e.g., eggs, pollen grains, and phytoliths) concentrations can be determined. During light microscopy analysis of the samples, the number of *Lycopodium* spores recovered is counted along with the numbers of microfossils observed. A microfossil concentration formula is then used to estimate the number of microfossils per gram of material as follows: Microfossil concentration $= [(p/m) \times a]/w$, where p is the number of microfossils counted, m is the number of marker grains (*Lycopodium* spores) counted, a is the number of *Lycopodium* spores added to the sample, and w is the total weight of the subsample prior to rehydration.

After dosing with dissolved *Lycopodium* spore tablets, samples were passed through a 250 μm mesh screen to separate macroscopic remains (material

larger than 250 μm) from microscopic remains (material smaller than 250 μm). Macroscopic remains were transferred to clean, appropriately labeled filter paper and allowed to dry.

The microscopic remains obtained following screening were concentrated into 50 ml plastic, screw-cap, graduated centrifuge tubes via repeated centrifugation. Examination slides of each sample were created by mixing a small amount of concentrated material with a drop of glycerin onto a clean, glass microscope slide and topping with a 22 mm × 22 mm cover slip. Slides were examined using a Nikon compound microscope. Any eggs encountered were photographed using a Sony Cybershot 18.2 megapixel camera and measured using an integrated ocular micrometer. Each egg was photographed and measured except in cases were eggs were damaged or folded and accurate measurements could not be obtained (Fig. 2). Subsequently, parasite egg concentration values were calculated for each of the 34 samples containing the eggs of *E. vermicularis*. All measurements are in microns unless otherwise noted.

The unprocessed half of each sample was saved in its designated secondary analysis bag and returned to the CMC collection of material in the Pathoecology Laboratory. Additionally, half of each processed subsample was archived and labeled as a preparation of parasite eggs using standard archaeoparasitological archival techniques. The other halves of the processed subsamples were preserved in 95% EtOH for future processing for palynological analysis.

RESULTS

Many of the eggs observed contained developed larvae, and microscopic analysis revealed evidence of *E. vermicularis* in 34/100 samples. The average egg concentration value for coprolites containing eggs was 157 eggs/g. Egg concentration values ranged from 36 eggs/g to 1,901 eggs/g. Concentration values expressed in eggs/coprolite rather than eggs/g ranged from 372 eggs/coprolite to 2,985 eggs/coprolite, with an average of 373 eggs/coprolite. Recovered eggs ranged in length from 49.0 to 63.7 and in width from 19.6 to 31.85. On average, the eggs recovered from CMC coprolites measured 56 × 29 (Morrow, 2016, unpublished dissertation, University of Nebraska–Lincoln, Lincoln, Nebraska, U.S.A.).

When accounting for all of the coprolites examined in the present study, a pattern of overdispersion with regard to egg distribution emerges (Figs. 1, 2). No eggs were recovered from 66% of the coprolites. Of the 34% that did contain the eggs of this parasite, the majority of coprolites presented with low concentrations of eggs, while only a few presented with high concentrations of eggs (Figs. 1, 2).

DISCUSSION

The results of the present archaeoparasitological analysis show that *E. vermicularis* was present among the Loma San Gabriel. Although a few of the eggs recovered were fractured, most of the eggs found among these samples were relatively well preserved. Photographs and measurements of recovered eggs were used to positively identify these eggs as *E. vermicularis*. The majority of the eggs fell within known parameters for *E. vermicularis*, which are 50–60 in length by 20–32 in width (Thienpoint et al., 1979), and those eggs that we observed that fell outside these parameters were only slightly outside of this range (i.e., 49 length or 19.6 width). These slightly smaller eggs could be the result of taphomomic changes as opposed to being authentically smaller in size (Morrow et al., 2016).

Given that a single gravid female *E. vermicularis* deposits approximately 4,600–16,000 eggs/day (Kliks, 1990; Ferreira et al., 1997; Roberts et al., 2012), these data indicate that the depositors of *E. vermicularis* positive coprolites were only lightly infected with these parasites (mean = 157eggs/g; range = 54–2,985 eggs/coprolite). However, the eggs of this parasite are light weight and easily aerosoled, and may adhere to surfaces within a residence. These eggs may be transmitted via inhalation or ingestion in this manner (Hugot et al, 1999), and individuals may also become autoinfected. Though eggs are deposited within the intestine (Garcia, 2009), they are not laid within the feces themselves (Caldwell, 1982). For this reason, only 5–15% of modern cases of enterobiasis report the recovery of eggs from fecal examinations (Cook, 1994; Brooker and Bundy, 2009). Thus, despite the low concentrations of eggs recovered in the present study as compared to the fecundity of modern pinworms, the recovery of these eggs from 34% of CMC coprolites suggests that much of the population utilizing the site likely was infected. This further tells us that CMC was either occupied regularly enough to sustain the transmission cycle among different groups of Loma San Gabriel using the site, or that those who used the site perpetuated the life cycle among one another at other, more permanent sites of residence. Parasites like *E. vermicularis* proliferate in human populations with

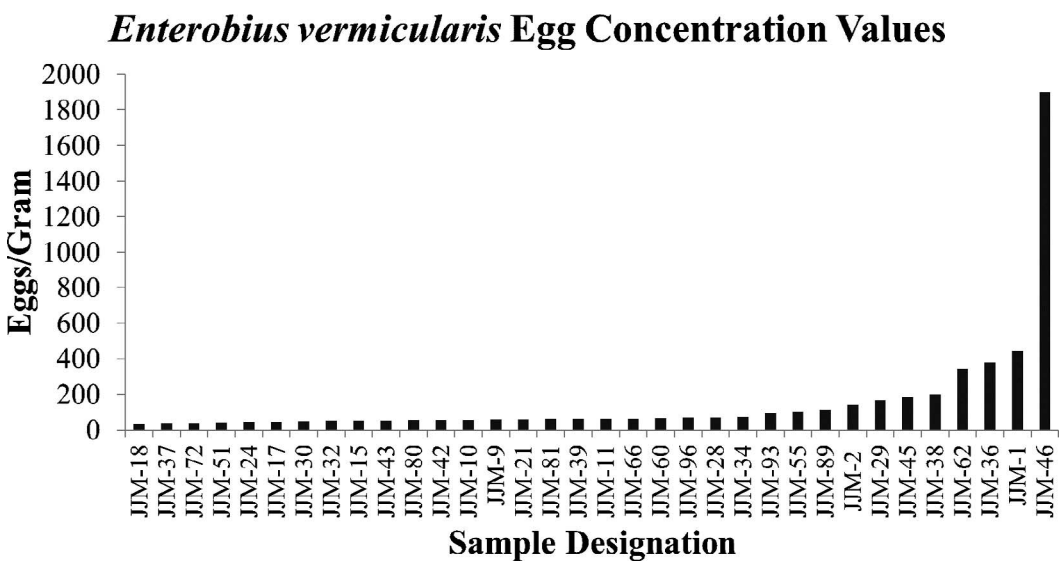

Figure 1. Egg concentrations from La Cueva de los Muertos Chiquitos coprolites infected with *Enterobius vermicularis*.

somewhat sedentary life styles. This is a parasite often associated with overcrowding that spreads easily within continuously populated domiciles. Though it is not known exactly how many individuals are represented by the 100 coprolites examined, an idea of the population profile was recovered from dental impressions of quids by Hammerl et al. (2015). Quids are expectorated masses of fibers that are common in archaeological sites used by people dependent on high-fiber plant resources. Analysis of 50 quids revealed dental impressions showing that the site was used by all age groups (Hammerl et al., 2015), with 49 distinct dental arcades from 49 people evident from the dental casts. These dental data suggest that a relatively large group of people, consistent with a permanent village, used the site. Additionally, 21 children younger than three years of age were buried at the site, which suggests that a larger population used the site. Given these data, it is estimated that the site was used by 100–250 people. In short, the life cycles of these parasites were being per-

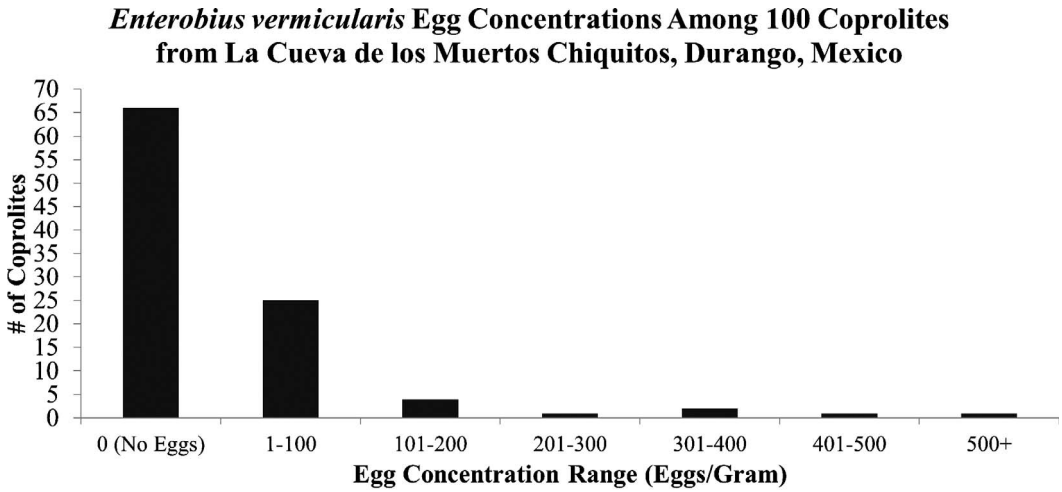

Figure 2. The concentrations and distribution of *Enterobius vermicularis* eggs among La Cueva de los Muertos Chiquitos coprolites.

petuated in those utilizing CMC approximately 1,300 years ago.

The overdispersed nature of the data presented herein is commonly encountered in modern parasitological systems (Crofton, 1971; Croll and Ghadirian, 1981; Crompton et al., 1984; Anderson and May, 1985; Shaw et al., 1998). While overdispersion is commonly discussed in reference to modern parasitism, it has rarely been discussed from an archaeoparasitological perspective. At CMC, the majority (66%) of coprolites did not contain *E. vermicularis* eggs, while the majority of the 34% of coprolites that did contain these eggs presented with very low egg concentrations, and only a small percentage of coprolites presented with high egg concentrations (Figs. 1, 2). Because it is not known precisely how many human individuals are represented by the 100 coprolites examined, it is not possible to definitively state that a negative binomial pattern of distribution of these eggs existed within this host population. Similarly, because many infected individuals do not pass the eggs of *E. vermicularis* in their stool, it is difficult to assess how many individuals were actively infected with this parasite. Future analyses of the remaining material from these coprolites utilizing aDNA extraction methods could shed light on the nature of *E. vermicularis* distribution among the Loma San Gabriel at La Cueva de los Muertos Chiquitos. Such future analyses should seek to assess the number of individual hosts represented by comparing human aDNA among the samples and should examine the coprolites for molecular evidence of *E. vermicularis* to more accurately assess the rates of infectivity among this population.

Antelope House is an archaeological site occupied around 900 years ago and was a permanent village constructed in a large rockshelter. Previous analysis of Antelope House yielded a 24% prevalence of *E. vermicularis* eggs among 180 coprolites (Hugot et al., 1999), a number similar to what we observed at CMC (20% prevalence of *E. vermicularis* eggs among 100 coprolites). Overall, Hugot et al. (1999) examined coprolites from 10 archaeological sites, dated from 8000 BC to 1300 AD, for the presence of *E. vermicularis* eggs. Their results indicate that only 1.8% of hunter-gatherer sites in caves or rock shelters were positive for pinworm eggs, and no pinworm eggs were recovered from cave sites without stone-walled villages, while cave sites with stone-walled villages presented with a prevalence of 19% among examined coprolites.

The results of the present study are similar to those reported by Jiménez et al. (2012), who examined 36 coprolites from CMC and found *E. vermicularis* prevalence to be 26% utilizing a flotation technique and 35% utilizing a sedimentation technique. By increasing the sample size to 100 CMC coprolites and using a standard rehydration, disaggregation, and screening technique, we report a 34% prevalence of *E. vermicularis* eggs among CMC coprolites. Combining the results of these studies, *E. vermicularis* eggs have now been reported from 50 of 136 CMC coprolites (36.8% prevalence among CMC coprolites).

The data presented herein expand our understanding of prehistoric human enterobiasis in the New World and are especially significant for establishing the paleoepidemiological patterns of pinworm infections among a transitional culture such as the Loma San Gabriel. Future analyses of both host and parasite aDNA extracted from CMC coprolites could further elucidate paleoepidemiological patterns.

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